European Journal of Biological Sciences 5 (2): 44-49, 2013
ISSN 2079-2085

DOI: 10.5829/idosi.ejbs.2013.5.2.7245

Evaluation of Natural Sources of Carotenoid Pigments from *Rosa rubiginosa* on Growth, Survival and Coloration of *Xiphophorus helleri* Fish Fry

[1]C. Arulvasu, [1]S. Ramya Meena, [1]D. Chandhirasekar and [2]S. Sivaganam

[1]Department of Zoology, University of Madras,
Guindy Campus, Chennai-600 025, Tamilnadu, India
[2]Central Institute of Brackish water Aquaculture, Chennai-600 028, Tamilnadu, India

Abstract: The present study was to investigate the effect of natural sources of carotenoid pigments from *Rosa rubiginosa* petals on growth, survival and coloration of ornamental swordtail *Xiphophorus helleri* fish fry. Homogeneous groups of swordtail fish fry were fed both a control (formulated diet) and three different supplement diet (including 200, 1000 and 2000 mg/kg petals extract), twice a day. The feeding experiment was continued for a period of 28 days. The experimental observation was carried out at seven days interval for 28 days. The coloration was enhanced in all the treated groups in respect with their diet concentrations but no change was observed in coloration of the control group. The maximum coloration was found in the treated group III with reddish orange tint from soft orange color. Specific growth rate (SGR) were significantly different among groups by the various dietary treatments ($P>0.05$). The maximum weight and length was recorded in treated group III with 0.30±0.01 g and 2.96±0.05 cm. The higher survival rate of 96.6±0.57% was recorded in the treated group III. The present study it could be concludes that the natural sources of carotenoid pigments extract from *R. rubiginosa* petals have a positive role in the growth, survival and colour development of fish fry.

Key words: *Rosa rubiginosa* · *Xiphophorus helleri* · Carotenoid · Pigmentation

INTRODUCTION

Ornamental fishes are rapidly gaining importance nowadays because of their aesthetic and immense commercial value in the export trade world over. Ornamental fishes are characterized by a wide diversity of colours, colour patterns and success in the ornamental fish trade is very much dependent on the bouncy colour of the fish. Color is one of the major factors, which determines the price of aquarium fish in the world market [1]. Attractive coloration determines the commercial value of ornamental fishes. Pigmentation in the skin is responsible for coloration in the fish. Carotenoids are the primary source of the pigmentation on the skin of fishes. In natural environment, the fishes meet their carotenoid requirements by ingesting aquatic plants or through their food chains. But, fishes cannot synthesize carotenoid *denovo*. Carotenoids are responsible for many of the red, orange and yellow hues of plant legumes, fruits and flowers. The colour enhancing diets should contain additional natural pigment to enhance the colour of the ornamental fishes.

Goodwin [2] established that fish do not possess the ability to synthesize carotenoids. The coloration in fish resulted from the pigment present in the diet [3, 4]. Many studies have proved that the fish can be pigmented by including processing wastes and plant sources [5-7]. Fishes display a variety of skin coloration patterns which are of great ecological, physiological and behavioral importance. According to Moyle [8], eight general color patterns can be described in fishes: (1) red coloration, (2) poster colours, (3) disruptive coloration, (4) counter shading, (5) eye ornamentation, (6) eye spots, (7) lateral stripes and (8) polychromatic. The sophisticated chromatic properties observed in this class of vertebrates provide protection from predators, advertising territories, assist in survivor and intraspecific communication [9].

Corresponding Author: C. Arulvasu, Department of Zoology, University of Madras, Guindy Campus, Chennai-600 025, Tamilnadu, India. Tel: +91-44-22202836.

Commonly used carotenoids in fish feed include natural and synthetic astaxanthin, cantaxanthin and lutein. Astaxanthin has been used widely as a potential pigmenting substance for both ornamental and farmed fish. NatuRose meal is a safe natural source of concentrated astaxanthin. The majority of the NatuRose carotenoid fraction is astaxanthin, with about 15% of the remainder consisting of canthaxanthin, lutein and beta-carotene. NatuRose is spray dried and formulated into a fine dark red powder and is currently used worldwide as a coloration and nutrition source for numerous species. It has been successfully utilized for pigmenting shrimp *(P. monodon* and *P. japonicus*), rainbow trout, Coho, Atlantic salmon, poultry eggs, Koi, sea bream (Tai), yellowtail and ornamental fish (marine and freshwater) [10]. Therefore, the present study was carried out to evaluate the natural sources of carotenoid pigments extract from *Rosa rubiginosa* on growth, survival and coloration of *Xiphophorus helleri* fish fry.

MATERIALS AND METHODS

Animal Collection and Maintenance: The freshwater swordtail, *Xiphophorus helleri* fish fry were purchased from Ornamental fish farm (Golden Galaxy), Kulathur, Chennai, Tamilnadu, India. The collected fish fry were brought to the laboratory in plastic bags with oxygenated habitat water. The fishes were acclimatized to the laboratory conditions for 15 days in disinfected 3L circular troughs. During acclimatization, fish fry were fed with formulated diet. Healthy and disease free fish fry, with average body weight of 0.04±0.00g were selected for further experiments.

Collection and Identification of *Rosa Rubiginosa:* Aerial parts of the plant *Rosa rubiginosa* flowers were collected from Kancheepuram during January and February, 2012. Plant material was identified and authenticated by examination of the morphological characteristics by a Botanist from CAS in Botany, University of Madras, Chennai, Tamil Nadu, India.

Extraction of Carotenoid Pigments from *R. Rubiginosa:* The extraction of carotenoid pigments was done following by the method of Raj Mohan and Ramaswamy [11]. Briefly, the petals of the *R. rubiginosa* flower were separated and dried under shade at room temperature (29±1°C) for about 7 days. The completely dried petals were powdered and sieve to get fine powder of petals. The acetone petals extract was obtained by immersing the petal powder in acetone for 24 hrs in Erlenmeyer flask. Five hundred gram of rose petal powder was immersed in 5L (1:10 w/v) of acetone for 24 hrs in an Erlenmeyer flask. After that extract was filtered with whatman No. 1 filter paper. The extract was concentrated using a vacuum evaporator at 45°C under low pressure. After complete evaporation of the solvent, the concentrated extract was collected and stored in a refrigerator for later use.

Table 1: Formulation of experimental diets.

Ingredients	Control g/kg	Treatment I g/kg	Treatment II g/kg	Treatment III g/kg
Brown fish meal	275	275	275	275
Shrimp head meal	100	100	100	100
Prawn meal	50	50	50	50
Wheat gluten	65	65	65	65
Wheat flour	200	200	200	200
Soya bean meal	100	100	100	100
Broken Rice	100	100	100	100
Fish oil	20	20	20	20
Vitamin	10	10	10	10
Minerals	40	40	40	40
Carotenoid pigments	-	0.2	1.0	2.0

Preparation of Fish Feed: The formulated diet was prepared according to Deng-Yu Tseng [12]. Ingredients such as brown fish meal, powdered fish head, squid meal, wheat gluten, wheat flour, soya bean meal, broken rice, fish oil, vitamin premise, mineral, binder (tapioca flour) and water were used to prepare the formulated diet. The feed formulation is given in Table 1.

Formulated diet was supplemented with graded levels of carotenoid pigments extract of *R. rubiginosa* flower. In total 4 experimental groups were used for the present study and were designated as control group (C) and treated groups (I, II and III). Control group was fed with a formulated diet without any supplement. Correspondingly, the treated groups I, II and III were fed with 200, 1000 and 2000 mg/kg of formulated diet with carotenoid pigments extract of *R. rubiginosa* flower.

Experimental Design: The fishes were maintained in plastic troughs of three litre capacity and they were categorised into four groups with one control group and three experimental groups. Each group were maintained in three replicates with twenty fishes per trough. The trough were covered well with nylon mesh and provided with appropriate aeration. The experiment was carried out for 28 days during the due course the control group fishes were fed with formulated diet without supplementation of carotenoid pigments and the experimental group fish fry were fed with formulated diet with supplementation of

carotenoid pigments in three different concentrations. The fish fry were fed twice a day with respective feed to apparent satiation, with the daily ratio being divided into two equal parts and fed during 10:00 and 18:00 hrs. The water in the troughs was replaced with fresh and clean tap water on alternative days. The uneaten feed and faecal matters were siphoned off every day.

Measurement of Growth and Survival: The growth measurements such as weight and length of the fish fry were recorded individually. Sample size and total biomass were optimized in the tank to adjust the amount of feed for each experimental group. At the end of 28 days experimental period, specific growth rate, percentage of survival and mortality [13] was calculated as given below

- SGR (%)=100×(ln final weight/length - ln initial weight/length)/Total duration of the experiment.
- Survival (%) = Present number of fishes/Total number of fishes×100.
- Mortality (%) = 100 - Percentage of survival.

Where, ln = logarithm

Observation of Coloration on Fish Fry: The fish fry (both control and treated) were photographed in a standardized light condition using a digital camera (Cannon Digital PC 1585).

Statistical Analysis: The data obtained from experimental groups were subjected to one way Analysis of variance followed by a post-hoc test (Duncan's multiple range tests) to determine the level of significance. The level of significance was set at $P < 0.05$. Statistical package of SPSS (version 11.5, USA) was used for statistical analysis.

RESULTS

Effect of Carotenoid Pigments on Growth of *X. helleri:* The initial weight of the fish fry was 0.04±0.00 g and after the experimental period of 28 days the final weight of the fish fry in the control group was 0.1±0.00 g and correspondingly, it was observed 0.19±0.00, 0.25±0.00 and 0.30±0.01 g in treated groups I, II and III. The mean weight of the control group was 0.006±0.00 g and also observed 0.14±0.00, 0.21±0.00 and 0.25±0.00 g in treated group I, II and III respectively. The weight gain per day was calculated 0.002±0.00 g in control group with 0.005±0.00, 0.007±0.00 and 0.008±0.00 g in treated groups I, II and III respectively. The difference in the weight gain per day between control group with the treated groups I, II and III are 0.003 g, 0.005 g, 0.006 g. The specific growth rate of the control group was 3.40±0.02 % and treated groups I, II and III was calculated 5.08±0.04, 6.31±0.39 % and 6.65±0.36 % (Fig. 1).

The initial length of the fish fry was measured 1.36±0.05 to 1.46± 0.05 cm and after the experimental period of 28 days the final length of the fish fry in the control group 2.0±0.10 cm and treated groups I, II and III was measured 2.23±0.05, 2.36±0.05 and 2.96±0.05 cm respectively. The mean length of the control group was 0.63±0.11 cm and was observed to be 0.85±0.70, 0.93±0.05 and 1.50±0.00 cm in treated groups I, II and III respectively. The length gain per day was calculated 0.01±0.00 cm in control group and 0.02±0.00, 0.03±0.00 and 0.05±0.00 cm in treated groups I, II and III respectively. The difference in the length gain per day between control group and treated groups I, II and III are 0.01, 0.02 and 0.04 cm respectively. The specific growth rate of the control group 1.36±0.23 % and treated groups I, II and III was measured 1.74, 1.79 and 2.50 % (Fig. 2). Statistical analysis of the formulated diet with carotenoid pigments showed changes in the total growth rate in the three different concentrations were significant when compared to the formulated diet without carotenoid pigments ($P<0.005$).

Enhancement in Survival Rate and Control over Mortality: The survival rate in control group was 76.6 % and in treated group I, II and III showed 90.0 %, 93.3 % and 96.6 % respectively. The maximum survival was observed in the fish fry of treated group III with 96.6 %. The minimum survival rate was observed in control group with 76.6 %. At the end of the experimental period of 28 days the mortality in the control group was 24.40 % and was observed to be 10.0, 6.7 % and 3.4 % in the treated group I, II and III respectively (Fig. 3). Statistical analysis of the formulated diet with carotenoid pigments showed changes in the survival and mortality in the three different concentrations were significant when compared to the formulated diet without carotenoid pigments ($P<0.005$).

Coloration on *X. Helleri* Fish Fry Analysis: The *X. helleri* fish fry were found in uniform orange colour before commencing the experiment. After the experimental period the fish fry in the control group were observed in the same colour without any change. The treated groups I, II and III were observed in an enhanced reddish tint in their body. Among the three

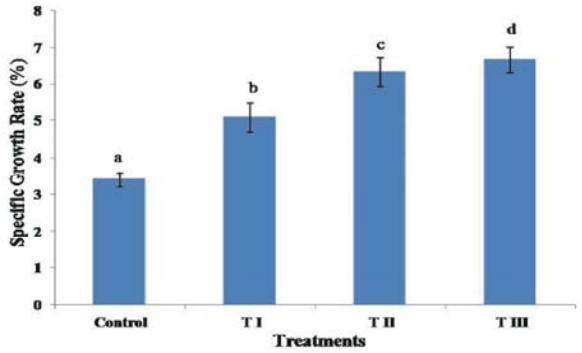

Fig. 1: Total body weight of swordtail *X. helleri* fish fry fed with and without carotenoid pigments for 28 days.

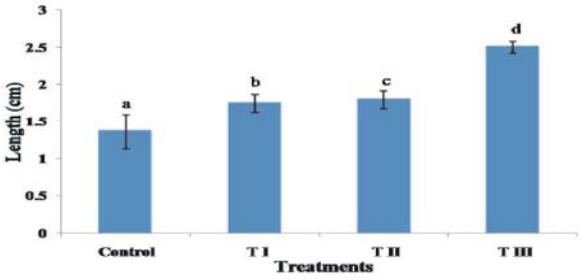

Fig. 2: Total body length of swordtail *X. helleri* fish fry fed with and without carotenoid pigments for 28 days.

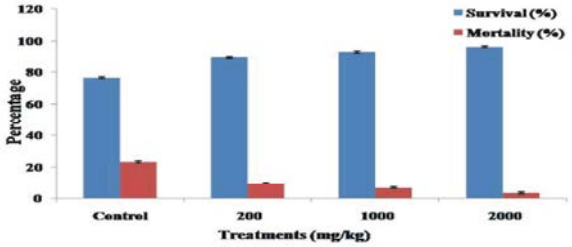

Fig. 3: Overall survival and mortality of swordtail *X. helleri* fish fry fed with and without carotenoid pigments for 28 days.

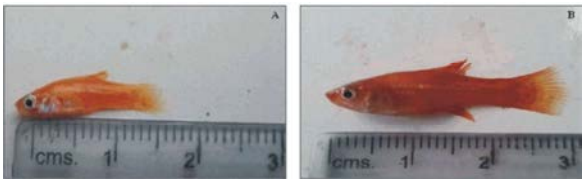

Fig. 4: Morphology of fish fed with and without carotenoid pigments for 28 days.

treated groups the fishes of the treated group III showed greater coloration compared to the other two treated groups. They were found in reddish orange colour at the end of the experimental period (Fig. 4).

DISCUSSION

Carotenoids are the primary source of pigmentation in ornamental tropical fish, responsible for various colours like yellow, red and other related colours. Normally these are obtained through substances rich in carotenoid content in the food chain of the aquatic organisms. But commercial feed ingredients such as yellow corn, corn gluten meal and *alfa alfa* are used as sources of carotenoids such as zeaxanthin and lutein [14]. Other carotenoids rich ingredients used are marigold meal (lutein), red pepper (*Capsicum* sp.) extract (capsanthin) and krill or crustacean meals (astaxanthin) [15, 16]. Carotenoids are also vital nutrients for healthy growth, metabolism and reproduction. The carotenoids do play a role in the growth of gold fish. Similar observation was made by Tveranger [17] in rainbow trout offspring using 10 percent krill meal as a source of carotenoid [18] stated that carotenoids influenced the growth of fishes and crustaceans.

Sommer *et al*. [19] recorded that in trouts, the addition of carotenoid rich micro algae *Haematococcus pluvialis* enhanced the growth of the fishes. Ronneberg *et al*. [20] also used *H. pluvialis* as a safe natural source of astaxanthin derived from micro algae which resulted in extensive pigmentation in koi and tropical fishes. Fey *et al*. [21] reported the enhancement of colour in male gourami when fed with carotenoid pigment source. Peimin *et al.* [22] reported that spirulina induced the growth and body colour of *crucian carp.* Boonyratpalin and Unprasert [5] observed a positive effect of dietary carotenoid on the growth of red tilapia. Dietary supplement of astaxanthin were found on the growth of Atlantic salmon (*Salmo salar*) reared for 22 weeks. Ezhil *et al.* [23] reported that feed with marigold petals increased the growth rate of Red swordtail (*X. helleri*) reared for 60 days. Phromkunthong [24] reported that the addition of spirulina (carotenoid source) was effective in producing deeper coloration in fancy carp. Sinha *et al.* [25] studied the growth rate of fishes in the group fed with the China rose petal feed was the highest in terms of weight, with an increased value of carotenoid in skin. The maximum carotenoid content found in 60 days fed group could be directly related to the enhanced level of carotenoid content in the particular ingredients.

Similarly, in the present investigation, *R. rubiginosa* incorporated feed in three different concentrations 200, 1000 and 2000 mg/kg were given to *X. helleri* fish fry for 28 days in which the treated group III fed with 2000 mg of carotenoid pigments mixed diet showed greater coloration.

The fish fry in the control group showed no colour difference and the treated group had a colour change of reddish orange from soft orange at the end of the experimental period.

According to the Tanaka *et al*. [26] in goldfish using crab waste as a carotenoid source and kamata *et al*. [27] who observed the colour development in rainbow trout when fed with *Adonis aestivalis* as a pigment source. Atlantic salmon juveniles with a mean weight of 1.75 g. were supplied with feeds with various levels of astaxanthin (0, 5.3, 36 and 190 ppm) for 10 weeks to study the effects on growth, survival and vitamin A content. It was found that the juvenile groups that had no astaxanthin supplied in the diet actually lost weight over the experimental period, while those with 5.3 ppm had a reduced SGR (specific growth rate) and BWI (body weight increase) compared to the higher doses. Groups fed the higher concentrations of 36 or 190 ppm of astaxanthin had the highest SGR, BWI and survival rates. The lipid content was also significantly higher in those groups fed as taxanth in at 36 and 190 ppm, while lower levels of astaxanthin resulted in fewer lipids and protein but a concomitant increase in moisture and ash content. The flesh astaxanthin and vitamin A content was found to be dose dependent. The results corroborate other studies that astaxanthin does indeed function as a pro-vitamin A source for juvenile Atlantic salmon with which body stores increase with dosage. It appears that the dietary needs for astaxanthin increases with the growth stage. Whereas the minimum levels for fry appear to be about 5.3 ppm, juveniles require a higher concentration as demonstrated by the feeds containing 36 ppm. It is apparent that astaxanthin has a specific role in salmonoid fry and juveniles linked to vitamin A metabolism and perhaps other functions. Stored astaxanthin from the flesh and skin apparently cannot be used as a vitamin A source; it must be converted at the point of uptake within the intestinal mucosa [28].

In the same way our study the maximum weight gains and higher growth rate was observed in the treated group III with 0.30±0.01 g and 6.65 %. The maximum length was obtained in the treated group III with 2.96±0.05. The control group showed the least length and weight gain when compared to the treated groups. Interestingly, a recent ground breaking study in Norway by Christiansen *et al*. [29] demonstrated that Atlantic salmon fry have a definitive requirement for astaxanthin in their diet for growth and survival. Fish fed with astaxanthin diet below 5.3 ppm were found to have marginal growth; those fed levels above 5.3 ppm had significantly higher lipid levels accompanied by lower moisture levels. When fry were fed astaxanthin concentrations below 1 ppm, the survival rates plummeted. More than 50% of the fry fed diet with less than 1.0 ppm astaxanthin died during the experimental period, survival of those groups receiving higher concentrations had survival rates greater than 90%. Thus, Atlantic salmon have the distinction as being the first salmonid species for which astaxanthin has been shown to be an essential vitamin, with minimum levels being about 5.1 ppm.

Regarding the survival and mortality in this study, the treated group III showed higher survival with lower mortality after the experimental period of 28 days. The present work confirmed that the carotenoid pigments plays a major role in induction of colour to the fish and is responsible for the increase in the growth rate and mortality. The Statistical analysis of both the formulated diet with and without carotenoid pigments showed changes in the total growth rate, survival and mortality in the three different concentrations were significant when compared to the formulated diet without carotenoid pigments ($P<0.005$).

REFERENCES

1. Saxena, A., 1994. Health; coloration of fish. International Symposium on Aquatic Animal Health: Program and Abstracts. Univ. of California, School of Veterinary Medicine, Davis, CA, U.S.A., pp: 94.
2. Goodwin, T.W., 1951. Carotenoids in fish. In: The biochemistry of fish. Bio. Chem. Soc. Symp., 6: 63-82.
3. Steven, D.M., 1948. Studies on animal carotenoids I. Carotenoids of the brown trout (*Salmo trutta* Linn.) J. Exp. Biol., 25: 369.
4. Hata, M. and M. Hata, 1973. Studies on astaxanthin formation in some freshwater fishes. Tohoku. J. of Agricultural Research, 24(4): 192-196.
5. Ibrahim, A., S. Shimizu and M. Kono, 1984. Pigmentation of cultured Red Sea bream Chrysophrys major, using astaxanthinfrom Antartic krill, *Euphausia superba* and a *Mysid, neomysis sp.* Aquaculture, 38: 45-57.
6. Boonyaratpalin, M. and W. Phromkunthong, 1986. Effects of carotenoid pigments from different source on colour change of fancy carp, *Cyprinus carpio* Linn. Songkhlankarin. J. Sci. Technol., 8(1): 11-20.
7. Ahilan, B. and Prince M.J. Jeyaseelan, 2001. Effects of different pigments sources on colour changes and growth of Juvenile *Carassius auratus*, J. Aqua. Trop., 16(1): 29-36.

8. Moyle, P.B. and J.J. Cech, 1988. Behavior and communication. In: Moyle, P.B and Cech, J.J. (Eds.), Fishes an Introduction to Ichthyology. Prentice Hall, New Jersey, pp: 148-166.
9. Fujii, R., 1993. Coloration and chromatophore. In: Evans, D.H. (Ed.), The Physiology of Fishes. CRC Press, Boca Raton, pp: 535-562.
10. Hempel, E. ,1997. Seafood International. pp: 29-31.
11. Raj Mohan, D. and M. Ramaswamy, 2007. Evaluation of larvicidal activity of the leaf extract of a weed plant, Ageratina adenophora, against two important species of mosquitoes, Aedes aegypti and Culex quinquefasciatus. Afr. J. Biotech., 6: 631-8.
12. Deng-Yu Tseng, 2008. Effects of stanniocalcin 1 on calcium uptake in zebra fish (*Danio rerio*) embryo. Am. J. Physiol. Regul. Integr. Comp. Physiol., 296: 549-557.
13. Rhcker, W.E., W.S. Randall, D.J. Brett and J.R. Hoar, 1979. Growth rates and models. In Fish physiology, Vol. 8. Bioenergetics and growth. Academic Press, New York, pp: 677-743.
14. Lovell, R.T., 1992. Dietary enhancement of colour in ornamental fish. Aquaculture Magazine, 18: 77-79.
15. Boonyaratpalin, M. and R.T. Lovell, 1977. Diet preparation for aquarium fish. Aquaculture, 12: 53-62.
16. Boonyaratpalin, M. and N. Unprasert, 1989. Effect of pigments from different sources on colour changes and growth of red *Oreochromis niloticus*. Aquaculture, 79: 375-380.
17. Tveranger, B., 1986. Effect of pigment content in broodstock diet subsequent fertilization rate, survival and growth rate of rainbow trout (*Salmo gairdneri*) offspring. Aquaculture, 53: 85-93.
18. Thongrod, S. A. Tansutapanich and O.J. Torrissen, 1995. Effects of dietary astaxanthin supplementation on accumulation, survival and growth in postlarvae of *Penaeus monodon* fabricus, Larvae 95 – Fish and shellfish larviculture symposium. Eur. Aqua. Socie. Special. Bull. 24. Gent. Belgium.
19. Sommer, T.R., F.M.L.D. Souza and N.M. Morrisssy, 1992. Pigmentation of adult rainbow trout *Oncorhynchus mykiss*, using the green alga *Haematococcus pluvialis*. Aquaculture, 106: 63-74.
20. Ronneberg, H., G. Borch, D. Fox and S.L. Jensen, 1979. Animal carotenoid 19, Natu Rose (Technical bulletin 054 Revision Date: 3/27/98).
21. Fay, M.F., M.D. Lledó, M.M. Kornblum and M.B. Crespo, 1999. From the waters of Babylon? *Populus euphratica* in Spain is clonal and probably introduced. Biodiversity and Conservation, 8: 769-778.
22. Peimin, H., Z. Yinjiong and H. Wenhui, 1999. Effect of the spirulina feed on the growth and body colour of crucian carp. J. Fish. China/Shuichan Xuebao., 23: 162-168.
23. Ezhil, J., C. Jeyanthi and M. Narayanan, 2008. Marigold as a Carotenoid Source on Pigment and Growth of Red Swordtail, *Xiphophorus helleri*. Turkish J. of Fisheries and Aquatic Sci., 8: 99-102.
24. Phromkunthong, W., 1988. Effect of carotenoid pigments from different sources on colour changes of fancy carp *Cyprinus carpio*. Abstracts of Master of Science Thesis (fisheries science), 1988. Notes Fac. Fish. Kasetsart Univ. Bangkok. pp:1.
25. Sinha, A. and Oyas Amed Asimi, 2007. China rose (*Hibiscus rosasinensis*) petals: a potent natural carotenoid source for goldfish (*Carassius auratus* L). Aquaculture Res., 38: 1123-1128.
26. Tanaka, Y., T. Katayama and K.C. Simpson, 1976. The biosynthesis of Astaxanthin-XIX. The distribution of - Doradexanthin and the metabolism of carotenoids in goldfish. Bull. Jap. Soc. Sci. Fish., 42: 885-891.
27. Kamata, T., G. Neamtu, Y. Tanaka, M. Sameshima and K. Simpson, 1990. Utilization of *Adonis aestivalis* as a dietary pigment source for rainbow trout *Salmo gairdneri*. Nippon Suisan Gakkaishi, 56: 783-788.
28. Christiansen, R. and O.J. Torrissen, 1996. Growth and survival of Atlantic salmon, *Salmo salar* L. fed different dietary levels of astaxanthin, Juveniles. Aquac. Nutr., 2: 55-62.
29. Christiansen, R., O. Lie and O.J. Torrissen, 1995. Growth and survival of Atlantic salmon, *Salmo salar* L. fed different levels of astaxanthin. First-feeding fry. Aquac. Nutr., 1: 189-198.